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Biological assay systems using indigenous holo- and meroplankton were designed to model the chlorination patterns of power plants. A matrix of chlorine concentrations and exposure patterns permitted the generation of response isopleths that were then applied to developing design criteria. The marine phytoplankter, Thalassiosira pseudonana showed a 50% reduction in photosynthesis when exposed to 0.15 ppm Cl_2 for 10 minutes, and complete growth inhibition after 5 minutes exposure to 0.3 ppm. Microzooplankton adults were somewhat less sensitive in that a 5 minute exposure at 2.5 ppm was necessary to produce 50% mortality. Larval and juvenile fish were sensitive to chlorine levels <0.2 ppm for exposure periods of sixty to ninety minutes.

Two field studies were evaluated and compared to laboratory data with specific emphasis on the use of ATP to monitor entrainment and damages. A review of pertinent literature is also included.

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June 1976

POWER PLANTS, CHLORINE, AND ESTUARIES

by

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ABSTRACT

Chlorine has and continues to be the principal biocide in systems using marine waters for cooling purposes. While considerable information exists on the role of chlorine applicable to specific fouling organisms, data on its impact upon non-target marine species is limited.

Results of a year's field investigations at a power plant on upper Narragansett Bay indicated that total residual chlorine at >1.0 ppm was responsible for complete mortality of all pumped phytoplankton and up to 75% of the zooplankton. In terms of biological impact, this amounted to approximately 15 tons of primary producer carbon and 1.6 tons of primary consumer carbon from June to December.

Biological assay systems using indigenous holo- and meroplankton were designed to model the chlorination patterns of power plants. A matrix of chlorine concentrations and exposure times permitted the generation of response isopleths that were then applied to developing design criteria. This data indicated that certain algal species had a 50% reduction in photosynthesis at 0.15 ppm after 10 minutes exposure and complete growth inhibition at 0.3 ppm after 5 minutes exposure. Microzooplanktonic adults showed 50% mortality after 5 minute exposures to 2.5 ppm total residual chlorine. Furthermore, chlorinated seawater showed residual toxicity to algae, 100 hrs. post-dosing, when no detectable residual chlorine was present.

Studies of larval and juvenile fish emphasize that short-term exposure to chlorine levels <0.2 ppm will produce a significant biological effect under routine intermittent dosage conditions.

Laboratory bioassay data was verified in a field study where the researcher was capable of modifying power plant operations. This study also was used to both develop and verify the use of ATP as a measure of pumped damage to both zooplankton and phytoplankton.

The above studies, supplemented by existing data on the toxicity of chlorine to marine organisms, have resulted in a modification of chlorination practices, more stringent effluent guidelines, and revised water quality criteria.

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INTRODUCTION

The importance of estuarine communities to man for both recreation and sustenance cannot be overemphasized. Estuarine productivity is estimated at 20,000 kCal/m^2/year compared with 1,000 kCal/m^2/year for the open ocean (1). Productivity alone, however, doesn't truly describe the importance of estuaries. What must be realized is that commercially important species of fish, shellfish, and crustaceans are dependent on the highly productive phytoplankton and zooplankton populations for food. In addition many of these same species of macrofauna are dependent upon the water column for spawning, early development, and growth during portions of their life history. McHugh (2) reports that 63% of the commercial catch of fish and invertebrates in the Atlantic consists of species dependent on estuaries. The National Estuarine Pollution Survey (3) notes that 65% of marine fish require the rich estuarine environment for some part of their life history. Thorson (4) estimates that 70% of all bottom invertebrate species have planktotrophic larvae that inhabit the water column for a 2-4 week period. Jeffries (5) reports that meroplankton comprise from 10-80% of the total zooplankton population collected from three East Coast estuaries.

The salient point from these studies is that the water column supports a myriad of planktonic forms that represent important life stages, from a wide variety of commercially and ecologically important communities. Yet, it is this very component of the estuarine system that is being used increasingly as a source of cooling water for a variety of industrial uses. This water contains a vast array of living forms which are then subjected to mechanical, thermal, and chemical stress. There are 140 existing or planned power plants located on marine sites, of which 133 have continuous flow, once-through cooling (6). The projected cooling water demands from these plants amount to 1×10^5 cfs (2.8×10^3 m^3/sec). This converts to a yearly consumption of 2.6×10^{13} gallons ($\sim 1.0 \times 10^{11}$ m^3).

With this projected usage of nearshore marine waters it is important that research effort be directed toward identifying the potential impact of this type of operation to the environment. Since marine life in water used for cooling purposes is subjected to three major stress components--mechanical, thermal, and chemical--an evaluation of the relative importance of these as related to biological impact is necessary. The objective of the study reported here was threefold--to examine an operating facility and focus on one major stress component (chlorine) in order to determine which portion of the biotic community is affected; to design and develop laboratory

programs that will determine the tolerance limits of a variety of organisms to this stress component; and finally to verify laboratory data in the field.

The results of these investigations will hopefully have multiple applications. The information can be used to guide the improvement of operational and design characteristics of power plants, with the objective of minimizing biological impact. The data will also contribute to an understanding of stress tolerance in biological systems and to the establishment of water quality criteria necessary for the continued vitality of estuarine communities.

CHLORINATION

Although the chemistry of chlorine as a biocide and disinfectant has been investigated for freshwater systems, particularly in regard to municipal sewage, less is understood of interactions in seawater. The following discussion is based upon knowledge obtained from chlorination of municipal wastes, but is applicable to understanding the general concepts of (7).

Chlorine gas, when dissolved in water, completely hydrolyzes to form hypochlorous acid (HOCl).

$$Cl_2 + H_2O \rightleftarrows HOCl + H^+ + Cl^-$$

At concentrations below 1000 mg/l and above pH 3.0, no chlorine exists in solution as Cl_2. The hypochlorous acid then dissociates to H^+ and OCl^- (hypochlorite). This dissociation is strongly pH dependent.

$$HOCl \rightleftarrows H^+ + OCl^- \text{ (hypochlorite)}$$

Being a strong oxidizing agent chlorine (HOCl) will readily react with a variety of organic and nitrogenous compounds. The most important reactions of chlorine with organic or inorganic nitrogenous compounds are described in the following equations:

$$NH_3 + HOCl \rightleftarrows NH_2Cl + H_2O \text{ (monochloramine)}$$

$$NH_2Cl + HOCl \rightleftarrows NHCl_2 + H_2O \text{ (dichloramine)}$$

$$NHCl_2 + HOCl \rightleftarrows NCl_3 + H_2O \text{ (trichloramine)}$$

The disinfecting capacity of chloramines is a function of the amount and rate of hypochlorous acid formation. The hydrolysis constant of monochloramine is too low for hypochlorous acid to be formed in significant amounts; dichloramine has a higher hydrolysis constant and is, therefore, more effective for disinfection.

Non-nitrogenous organic compounds will also react with chlorine to produce toxic substances. The mono-, di-, and trichlorophenols have demonstrated toxicity to aquatic organisms (8). Jolly (9) has prepared an extensive review of chlorinated organics found in domestic sewage effluents.

The addition of chlorine, therefore, can result in a variety of chemical species being formed; the relative proportions of which are dependent upon pH, qualitative and quantitative composition of nitrogenous compounds, and other organic material present in the water. Such a potential complexity of biologically active and inactive chemical species necessitates some type of classification.

Free residual chlorine is the residual chlorine existing in the water as hypochlorous acid (HOCl) and hypochlorite ion (OCl^-). The disinfection capabilities of these two forms are quite different. Because of its neutral charge and small molecular size HOCl readily penetrates cell membranes and may exert its effect biochemically rather than as an oxidizing agent. The OCl^- ion, bearing a negative charge, is impeded in membrane passage. Of further biological significance is the pH dependent distribution of these forms in aqueous systems. In the pH range 6.5-7.2 (characteristic of freshwater), the dominant form is the more toxic HOCl. While at estuarine pH's 7.5-8.2 the reverse is true (7).

Combined available residual chlorine is that residual chlorine existing in water in chemical combination with ammonia and organic nitrogenous compounds. While having a lower oxidation potential than free chlorine forms, the chlorine is still available for chemical reactions. There are reports that chloramine toxicity to coho salmon and rainbow trout (10) was of the same magnitude as free residual chlorine. Arthur and Eaton (11) have shown the chloramine 96-hr TLm to Gammarus pseudolimnaeus and Pimephales promelas to be 220 µg/l and 85-154 µg/l, respectively. Concentrations producing no significant long term effects on these species were <3.4 µg/l for the amphipod and 16.5 µg/l for the minnow. As in the case of free residual chlorine, the distribution of mono- and dichloramines in aqueous systems is strongly pH-dependent (7). At seawater pH's only 20% of the total chloramine is present as the toxic dichloramine.

FOULING

With the widespread increase in the use of marine waters for cooling purposes the problems associated with fouling are increasing proportionally. The following comments are in no way to be construed as a review of the subject but rather to describe the situation that has resulted in the widespread use of chlorine as an antifouling agent.

Among the marine organisms often classified as major fouling types, the marine borers and mussels appear to be the most abundant and difficult to control. To further place the problem in perspective consider that studies at a Northeast Coast power station reveal an annual crop of 1000 tons/acre/year (220 kilograms/m^2/year) of the fouling mussel, Mytilus edulis (12).

Control methods for marine fouling organisms include: properly located revolving screens, predetermined increments in water velocity, use of elevated temperature, and the chlorination of intake water. Recently, combinations of temperature and chlorine have been investigated as have: mechanical condensor scouring systems (i.e. Amertap) and anodic introduction of copper and other biocides.

Early studies by White (13) detail the effect of elevated temperatures with and without additions of chlorine on the survival of Mytilus edulis. These studies were done on organisms from Lynn Harbor with continuous chlorination at 0.3 to 3.0 ppm for temperatures above 85°F (29.5°C) and with intermittent chlorination at lower temperatures. For this group of test organisms, temperatures >84°F (>29.0°C) the addition of chlorine did not appreciably decrease the time necessary to kill the mussels.

Mussels, unlike other molluscs, possess a gland in their foot which secretes a thread used to anchor the organism in place. This byssus gland appears to be particularly sensitive to chlorine. Clapp (14) investigated the effects of various chlorine concentrations and exposure regimes on the ability of Brachydontes exustus to remain attached to a substratum. His results indicated that continuous chlorination at 0.25 ppm-0.50 (nominal) was sufficient to cause all exposed mussels to loose their holdfasts and their shells to open. Intermittent chlorination of 2 hr duration, at 1.5 and 3.0 ppm with 2 hr off and 6 hr off also resulted in loss of attachment but not death. Return to undosed water after six days exposure revealed that the continuously dosed organisms did not recover while those with intermittent doses did recover and reattach. In addition, slime, barnacles, and Bryozoa were totally eliminated by continuous but not by intermittent chlorination.

Richards (12) using an approach similar to that of the Clapp studies, found that 0.25 ppm chlorine or a temperature of 110°F for 30 minutes/week was effective in preventing colonization by marine borers. Intermittent chlorination at 1.00 ppm for 1 hr/12 hrs in combination with 110°F for 30 minutes/week was also effective whereas chlorine alone in the above dosing regime was not effective.

Turner (15) found that continuous chlorination at 0.25 mg/1 prevented fouling over a 90 day interval, yet intermittent treatment at 10 mg/1 for 8 hrs per day was ineffective. James (16) found that

detachment and movement of mussels in the direction of water flow could be achieved with 0.02-0.05 mg/l residual chlorine levels.

Although various methods for fouling control have been documented for over two decades the practice has been to adopt chlorination as method of choice. Interestingly, whereas continuous low level application has been shown to produce most effective control, the usual practice in the U.S. is to use high intermittent doses. Further, until recently, there has been little serious effort to develop alternatives. This also seems unusual since the use of recirculating warm water was effectively developed as a primary control at Redondo Beach twenty years ago. Interest in this type of system, particularly for condenser slime control, is increasing. Several northeastern power companies are exploring short duration, high temperature condenser cycles with good success.

As a result of the widespread use of chlorine in fouling control by power companies, it was inevitable that concern would develop about the impact on non-target species. The burgeoning energy needs of the post-war era has resulted in the proliferation of generating facilities particularly in coastal areas. The large volumes of needed cooling water were more readily available from the marine environment than from the already overburdened fresh surface waters. By 1980 it is estimated that close to 2.5×10^{13} ($9.5 \times 10^{13} m^3$) gallons of cooling water will be taken yearly from estuarine waters (6). More than 70% of this will have to be treated to reduce fouling problems in some manner. It is imperative, therefore, that a data base be prepared that will allow evaluation of the potential impact of chlorine to non-target marine and estuarine waters.

FIELD ASSESSMENT

Field investigations at a 650 MW fossil fuel electric generating facility on upper Narragansett Bay were initiated in May 1970 and terminated in June 1971. At the time of this study, the cooling water demand was 6.5×10^5 gpm ($2.4 \times 10^3 m^3$/minute): a $\Delta T = 10^{\circ}C$; and a daily chlorination cycle of 30 minutes/each of three units/day. Dosing was as NaOCl at an initial concentration of ~10 ppm. Residuals in the discharge canal ranged from 0.5-3.0 ppm.

The objectives of this program were to develop sampling techniques for holo- and meroplanktonic forms which would permit the assessment of thermal, mechanical, and chemical effects to pumped organisms. Biological information was then integrated with plant operating modes and water quality data.

Sampling consisted of weekly collections from intake canal and discharge canal for both chlorination and non-chlorination cycles as well as from a control station in upper Mt. Hope Bay that was not

influenced by the plume. In order to reduce sampling variability, collections made at the intake and discharge stations were time phased to assure that the same water masses were being compared.

PHYTOPLANKTON

Water samples were collected with a 5 liter non-metallic Van Dorn sampler from a three meter depth. Four casts were made at five minute intervals and pooled. The pooled sample was mixed and two liters were placed in a thermos bottle for transportation to the laboratory. Replicate 25 ml aliquots were preserved in 1% glutaraldehyde for a microscopic examination. Triplicate 250 ml aliquots were immediately filtered, (~10 psi) frozen over silica gel in the dark, and analyzed for chlorophyl-a and phaeophytin (17). For productivity measurements, incubation facilities were set to simulate ambient temperature and light intensity. Six 125 ml flasks containing 50 ml of water were incubated with $Na_2{}^{14}CO_3$ (0.1 $\mu c/\mu g^C$ final sp. activity) for 12 hours. Four were incubated in the light while two incubated in the dark. Cellular radioactivity was measured by liquid scintillation spectrometry.

The results of chlorophyl-a analysis (Table 1) indicate that the three stations showed highly significant between station variation, when subjected to analysis of variance ($F = 4.68$; $P = 0.05$). Subsequent examination using Duncan's Multiple Range Test revealed intake and discharge without chlorination were homogenous subsets but that discharge with chlorination was not. While chlorophyl-a was somewhat lower in the discharge due to mechanical and thermal stress these differences were not significant. The effect of chlorination on the other hand is dramatic. On many occasions chlorophyl was not even detected in the discharge samples implying total loss of primary producer biomass. Using plant characteristics and assuming worst case conditions of total kill during the 90 minute/day chlorination cycle, the loss of primary producer carbon from the food web is ~16 tons (1.45×10^4 kilograms) of carbon from June-December.

Productivity data (Table 1) further implicates chlorination as the primary source of biological effect. The data substantiates the fact that thermal and mechanical stress have little or no impact on the phytoplankton during the summer-fall months. The interpretation of C-14 uptake data as obtained in this study is somewhat different from that of *in situ* studies where short term effects are measured. Since the photosynthetic system is often subject to transient effects it can be dangerous to extrapolate to longer term responses. Carbon-14 uptake as used in this study reflects more permanent effects since the 24 hr incubation time allowed for recovery to occur.

Table 1. ANALYSIS OF CHLOROPHYL-A AND PRODUCTIVITY DATA COLLECTED FROM INTAKE AND DISCHARGE FOR BOTH CHLORINATION AND NON-CHLORINATION OPERATIONAL MODES

Treatment	Mean	Std. error	N	F(0.05)[a]
Chlorophyl-a				
Intake	9.22	1.26	25	4.688
Discharge	7.72	1.23	25	
Discharge + Cl_2	3.41	0.87	14	
Productivity				
Intake	39.99	1.45	8	58.00
Discharge	36.91	2.18	8	
Discharge + Cl_2				

[a]Duncan's Multiple Range (Alpha 0.05). Intake and discharge are homogeneous subsets. Chlorinated discharge is significantly different.

Microscopic examination indicated that during periods of the year when chain forming diatoms such as Detonula confervaceae and Skeletonema costatum were at peak abundance there was extensive fragmentation of these chains. In addition, thermal stress was noted during March 1970 when Detonula was dominant. This species has an upper temperature range of 10-12°C. Since ambient water temperatures were at this level the resulting thermal load (10°C) stressed this species as was indicated by markedly depressed rates of photosynthesis. Such a phenomenon is likely to occur for many species during transitional periods of both spring and autumn if exposure times in the plume are long enough.

In summary these studies indicate that chlorination as practiced at this plant was responsible for severe damage to primary producer populations. Only under the specifically mentioned circumstances was either thermal or mechanical damage observed.

ZOOPLANKTON

Zooplankton samples were collected for the same sample dates and

stations described above. Sampling employed a modified Clarke-Bumpus sampler, equipped with a special "cod-end" bucket to reduce damage, a calibrated TSL flowmeter, and #10 mesh netting (153 micron apperture). Tows were of varying duration (1-15 minutes) depending on organism density and current speed. Intake samples were taken from the three meter depth while discharge samples were taken from the surface. The discharge canal (300' x 25' x 10') had a mean flow of 5'/sec (1.52m/sec) and was extremely turbulent and well mixed. The tow samples were diluted with ambient seawater and treated as follows: a portion was preserved in buffered formalin (10%) for microscopic examination and the remainder placed in insulated containers and transported back to the laboratory. In the laboratory, aliquots were diluted in two liters of filtered seawater. Since damaged or dead organisms sink faster than living, those organisms settling to the bottom and unresponsive to touch were counted as dead and preserved. The samples were then placed at ambient temperature for twenty-four hours and examined to determine living and dead. Total mortalities were tabulated and the remaining living organisms were preserved and counted. The data is presented as percent mortality (Fig. 1).

Chaetognaths and fish larvae were unable to survive the mechanical stress of plant passage. Microscopic examination revealed extensive fragmentation of these forms. Fish adults showed extensive mortality in the discharge area only during periods of chlorination. These organisms are attracted into the discharge area by the warm water and are unable to escape when the chlorination cycle is initiated.

Zooplankton populations are dominated by Acartia tonsa from June to October and Acartia clausi from November to May. During periods of chlorination (1-3 ppm total residuals) there is essentially complete mortality of Acartia tonsa. During non-chlorination periods these organisms appear to survive plant passage. There are moderate mortalities of Acartia clausi during April and May due primarily to the thermal stress. This situation is analogous to that described for Detonula confervaceae. Acartia clausi will not tolerate prolonged exposure to temperatures >15°C. Therefore when ambient temperatures reach 10-12°C, the additional rise of 10°C produces thermal stress above its tolerable limit.

From data on the population densities of Acartia tonsa and Acartia clausi during June-December (17) and assuming total mortality during chlorination periods, an estimated 1.6 tons (1.45×10^3 kilograms) of primary herbivore biomass was destroyed.

From these field studies it was obvious that estuarine populations of primary producers and primary herbivores were acutely sensitive to chlorine.

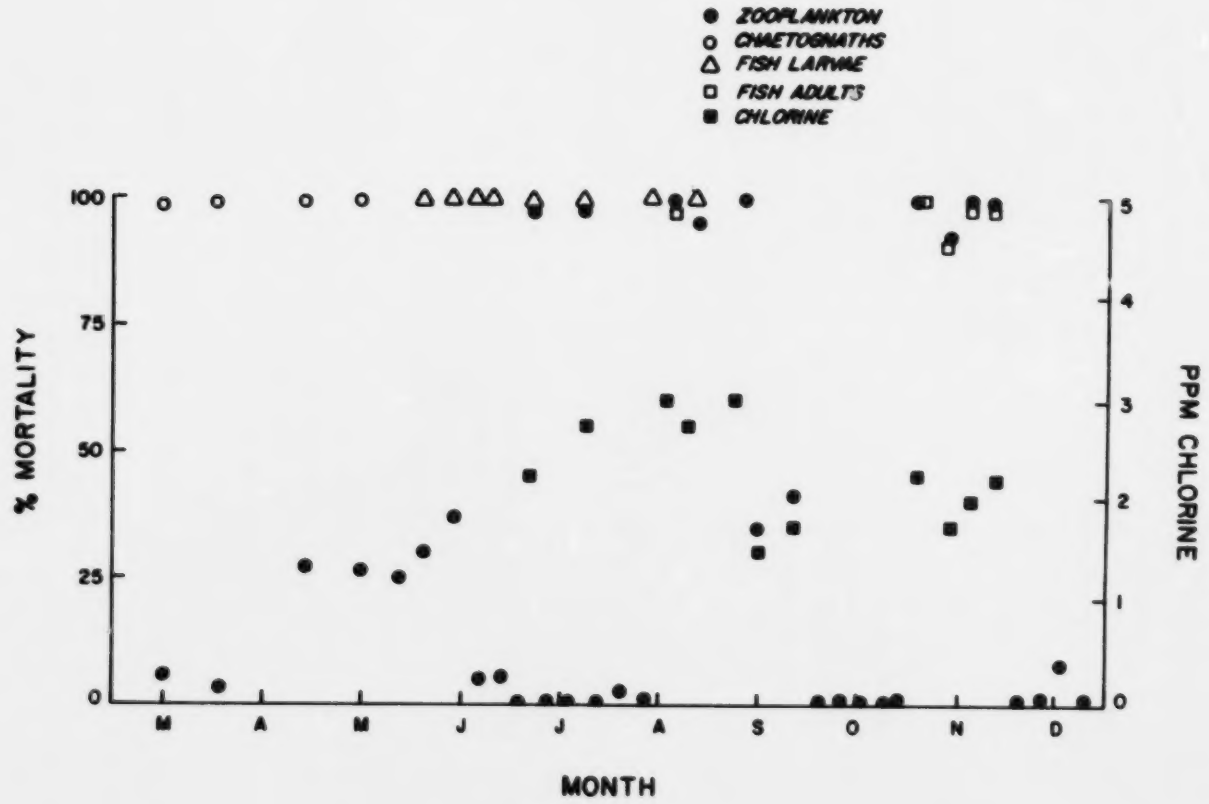

Figure 1. Seasonal distribution of mortality to entrained zooplankton at Narragansett Bay power plant

BIOASSAY MODELING

Literature describing the toxicity of chlorine to marine organisms is primarily related to fouling communities. Only limited information on ecologically important or indigenous species was available when this study was initiated. Since our staff has experience in both the culture and bioassay of holo-, mero-, and ichthioplankton, which are the major pumped species, a series of experiments was designed to simulate the effects of chlorine during passage through the plant and in the plume. A matrix of chlorine concentrations and exposure times was selected for each species and post exposure responses were measured periodically. Chlorine dilutions were prepared in filtered seawater (30 o/oo). Test species were exposed for varying periods of time and chlorine action was stopped either by the addition of sodium thiosulfate or transfer of the test species to clean seawater. Post exposure responses of growth, photosynthesis, and mortality were monitored for 48-96 hrs. Thiosulfate was shown to have no effect on the organisms at the levels used.

PHYTOPLANKTON

Axenic cultures of marine phytoplankton obtained from our culture collection were selected to include the primary ecological dominants from both winter and spring. Also included were food organisms often used in mariculture.

Studies were performed in enriched synthetic seawater (30 o/oo), 2500 lux continuous cool white illumination, at either 10° or 20°C. Growth rates (k) were determined from daily cell counts using an electronic particle counter, and the expression

$$k = \log_2 \frac{Nt}{(No)} \div t_t - t_o.$$

Rates of photosynthesis were determined immediately after dosing ceased by labelling an aliquot of the culture with $Na_2{}^{14}CO_3$ (0.1 µCi/2.4 µMc/ml). Triplicate light and duplicate dark exposures were incubated for four hours, filtered at <-5 psi, the filters were exposed to HCl fumes for 60 seconds, and the assimilated radioactivity was counted by liquid scintillation spectrometry.

Since there was no reference data on the sensitivity of marine phytoplankton to chlorine, routine short term (24 hr) bioassays on eleven species of algae at two temperatures were performed. The results (Table 2) indicate that these species are very sensitive to chlorine. The 24 hr LC-50 values were generally one-tenth the concentrations monitored in the discharge canal and one-one hundredth of that at the maximum initial dose. No attempt was made to monitor

Table 2. THE TOXICITY OF CHLORINE TO SELECTED SPECIES OF MARINE PHYTOPLANKTON
(Values are µgs Cl_2/liter that produced a 50% reduction
in growth rate during a 24-hour exposure period.)

Species[a]	24-hr IC-50 µgs/Cl/l	Species[b]	24-hr IC-50 µgs/Cl/l
Skeletonema costatum	95	Chaetoceros decipiens	140
Rhodomonas baltica	110	Thalassiosira nordensholdii	195
Dunaliella tertiolecta	110	Thalassiosira rotula	330
Monochrysis lutheri	200	Asterionella japonica	250
Thalassiosira pseudonana	75	Chaetoceros didymum	125
		Detonula confervacea	200

[a]Temperature 20°C.

[b]Temperature 10°C.

the chlorine levels in the test vessels. The original stock solution (100 ppm) was checked by titration with sodium thiosulfate and appropriate dilutions were used for lower levels. In order to determine if significant amounts of chlorine were lost during the twenty-four hour period, seawater dosed at 5 ppm was monitored every four hours. After twenty-four hours this value decreased to 4.3 ppm. In subsequent short term bioassays (<4 hrs) the problem of chlorine loss was not deemed significant for this system.

The concentrations of chlorine found toxic after twenty-four hours exposure are lower than those found to be satisfactory for antifouling control. Hydrographic conditions, however, are generally not favorable to prolonged exposures in power plants unless extensive cooling or discharge canals are present. Though informative, the twenty-four hour toxicity studies described above do not reflect actual in situ exposures and therefore are not predictive for most operating conditions.

The primary objective of subsequent studies was to examine a spectrum of chlorine concentrations and exposure times in an effort to delineate the precise relationship between these factors as they relate to biological response.

The model developed and described below was applied to four species of phytoplankton--Detonula confervaceae, Asterionella japonica, Skeletonema costatum, and Thalassiosira pseudonana. The most extensive series was performed on T. pseudonana and will be described in detail.

The test species was cultured in unenriched filtered natural seawater collected from the intake. This water contained adequate nutrients to support excellent growth without additional nutrient supplementation. Chlorine (NAOCl) was added to a growing culture of the test species (~5000 cells/ml) and the exposure time with a stopwatch. Sodium thiosulfate, which was used to stop the action of chlorine, did not interfere with growth or photosynthesis of the test species at levels one-hundred times greater than the concentrations employed. Post exposure aliquots were removed and incubated for four hours with $NaCl^{H}CO_3$. The remaining cultures were placed in an incubator and the growth rates measured for 48 hours to evaluate permanent damage (Table 3).

The shortest exposure interval was 10 seconds due to manipulative restrictions. At 1.0 ppm residual chlorine no growth was measured even at the shortest exposure, although there was some photosynthetic activity during the first four hours post exposure. At 0.5 ppm there were significant decreases in both photosynthesis and subsequent growth after only 15 seconds exposure with no growth recorded after 30 minutes exposure. As the concentration of chlorine decreased, longer exposure periods were required to elicit

Table 3. THE POST EXPOSURE 48 HOUR GROWTH RATES OF THALASSIOSIRA PSEUDONANA
(The values in parenthesis are the immediate effects on photosynthesis as %-control.)

Exposure-Seconds	Chlorine (mgs/1) 1.0	0.5	0.4	0.3	0.2	0.15
Control	1.92 (100)	1.82 (100)	2.44 (100)	2.96 (100)	2.70 (100)	2.42 (100)
10	0.06 (16)	1.55 (68)	2.30 (79)			
15		0.90 (21)	2.30 (67)	2.95 (100)		
20	0.03 (14)	0.55 (17)	2.20 (65)			
30	0.03 (13)	0.01 (14)	2.20 (58)	2.75 (100)	2.60 (99)	
60	N.G.[a](7)	N.G. (6)	1.26 (30)	2.83 (88)	2.50 (90)	
150			N.G. (24)	0.96 (25)	2.70 (68)	
300				N.G. (0.0)	2.60 (70)	2.60 (100)
600					2.10 (64)	2.30 (62)
1200					1.10 (33)	2.10 (48)

[a]N.G. indicates no measurable growth.

a response. At 0.15 ppm only moderate growth inhibition was recorded even after twenty minutes exposure, but initial photosynthesis was markedly reduced (48% of control). It is interesting to note that in several instances there are significant decreases in photosynthesis that are not reflected in corresponding decreases in growth rate. There was not enough data to thoroughly explore the relationship between photosynthesis and growth under chlorine stress. The data does indicate that information based solely upon C-14 assimilation could be misleading if extrapolation to long term affects were to be attempted.

The results of this study predict that chlorine concentrations >0.5 ppm cannot be tolerated by pumped phytoplankton during the usual 1-3 minute plant transit time. Survival and growth at 0.2 ppm is normal after five minutes exposure and only 26% inhibition is noted after a 20 minute exposure. It would appear that continuous chlorination at 0.2 ppm for anti-fouling purposes while having some impact would not seriously alter the productivity of this trophic level. The practice of intermittent chlorination at >1.0 ppm will essentially destroy all phytoplankton pumped during the chlorination cycles.

From the type of data discussed in Table 3, extrapolated values for zero, 25, 50, and 100% inhibition of growth can be calculated for specific exposure time at each chlorine concentration. The isopleths of response can then be graphically displayed (Fig. 2). Similar, though less detailed, data was generated for other species. Asterionella japonica exhibited sensitivity similar to that of T. pseudonana but Detonula confervaceae was 2-5 times less sensitive at concentrations <0.5 ppm. Skeletonema costatum (Fig. 3) was somewhat less sensitive to chlorine than Thalassiosira. S. costatum, however, is more sensitive to thermal stress than Thalassiosira with marked inhibition of growth at 32.5°C. Therefore, S. costatum would be expected to have a higher response interaction than Thalassiosira which can tolerate thermal stress exposures at 35-36°C. The graphical representation of response isopleths can be effectively used by design engineers since it gives precise and easily interpretable information on the degree of biological impact that will result from changes in operational mode.

ZOOPLANKTON-MEROPLANKTON

The approach as described above was employed with selected species of estuarine adult copepods and sand shrimp larvae. All copepod experiments were performed in filtered natural seawater, 30 o/oo at 15°C. Larval experiments were done at 10°C. The response measured was mortality 24 hours post exposure.

A detailed study was performed on Acartia tonsa because it is one

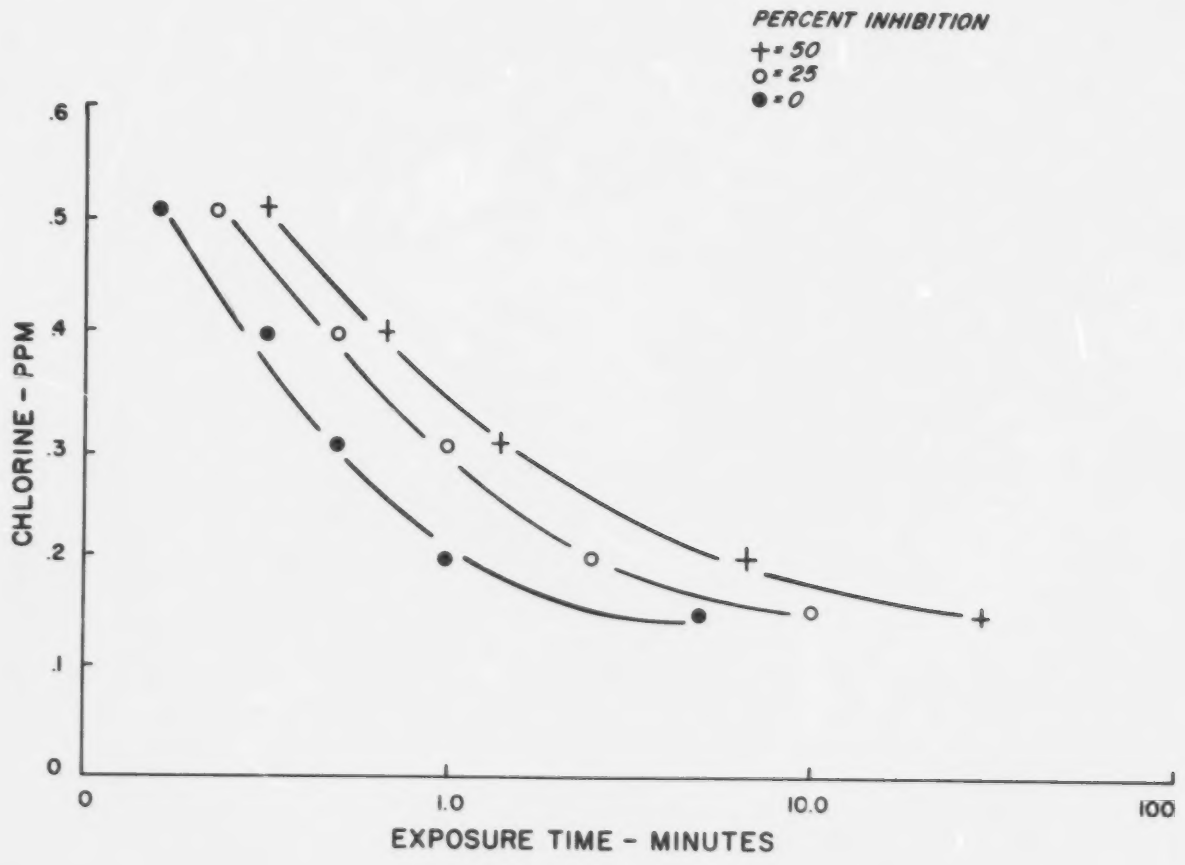

Figure 2. Response isopleths for the marine diatom, Thalassiosira pseudonana, exposed to chlorine

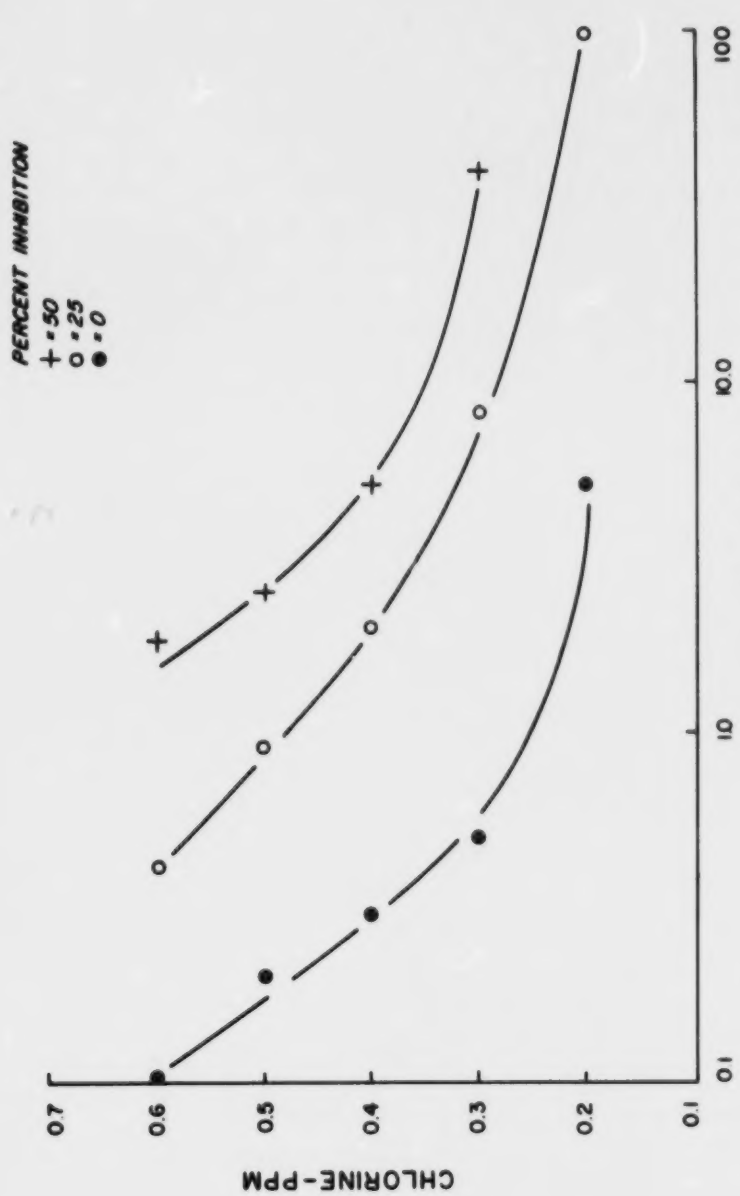

Figure 3. Response isopleths for the marine diatom, Skeletonema costatum, exposed to chlorine

of the most ecologically important primary herbivores. The results (Fig. 4) indicate this species to be much less sensitive to chlorine than the algae but still unable to tolerate chlorine levels above 1.0 ppm without substantial mortality. This study did not attempt to evaluate the potential long term effects of these exposures on subsequent survival and reproduction, not the combined effects of thermal addition and chlorine.

McLean (18) in a study designed to model the effects of the Chalk Point Power Station exposed A. tonsa at 6°C to 2.5 mgs chlorine/l for 5 minutes. A consistent mortality at 3 hours post exposure of 90% was recorded. We noted for identical exposure conditions at 15°C a mortality of 49%. Differences could be related to salinity, temperature, or pH. Of further interest is the occurrence of A. tonsa, apparently in abundance, at 6-8°C. Our experience would question this, as A. clausi is the usual congeneric cold water form which is abundant in the Patuxent River.

Additional species of copepods, Eurytemora affinis and Pseudodiaptomus coronatus were somewhat less sensitive than Acartia (Table 4).

Table 4. THE ACUTE TOXICITY OF ESTUARINE COPEPODS AND FISH LARVAE TO CHLORINE[a]

Species	Chlorine conc (mgs/l) 1.0	2.5	5.0	10.0
Acartia tonsa	120.0	5.0	2.0	0.7
Eurytemora affinis	360.0	9.0	4.0	2.0
Pseudodiaptomus coronatus	--	45.0	9.8	5.0
Pseudopleuronectes americanus	--	15.0	2.5	0.3

[a]Tabular values are exposure time (minutes) to produce 50% mortality.

Studies on the larvae of sand shrimp (Crangon septemspinosis) indicated this organism to be resistant to chlorine. At 10 ppm, 60% mortality resulted from a 5 minute exposure and at 5 ppm, 42% mortality was produced from a 10 minute exposure. These results were almost identical to those reported for Pseudodiaptomus coronatus (Table 4). McLean (18) reported that barnacle larvae

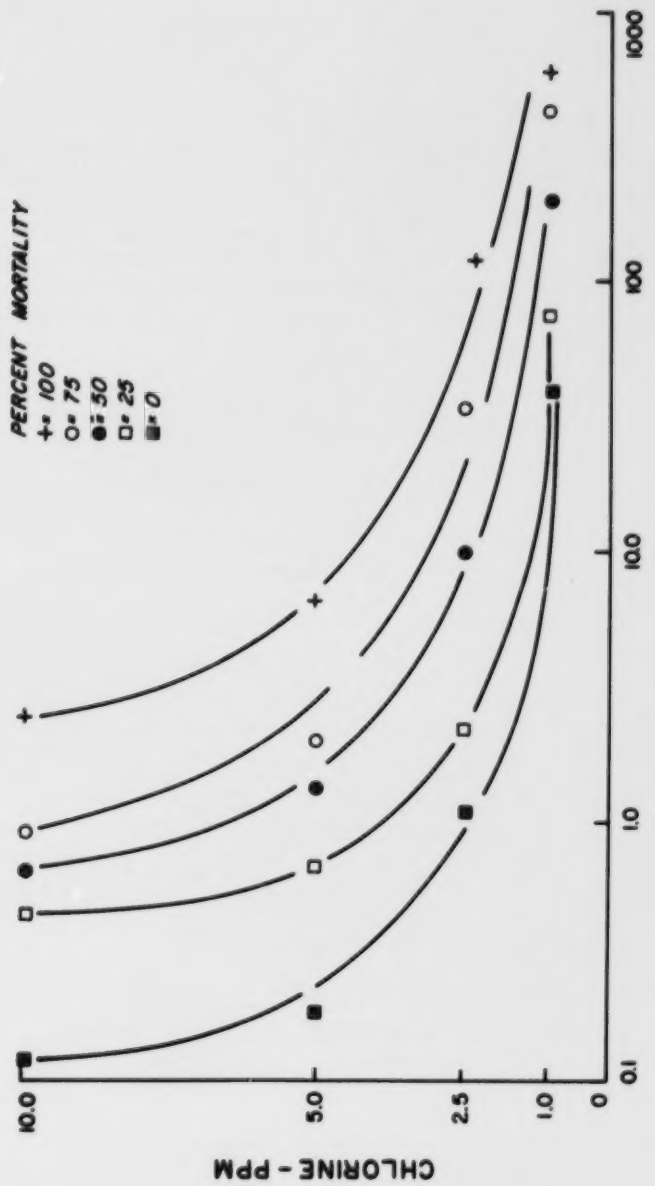

Figure 4. Response isopleths for the marine calanoid copepod, Acartia tonsa, exposed to chlorine

suffered approximately 75% mortality after 5 minute exposure to 2.5 ppm while similar exposures to the grass shrimp, Paleomenetes pugio, the amphipods, Melita nitida and Gammarus sp., showed essentially no mortality.

While not as sensitive as the microalgae, specific species of microzooplankton do show extensive damage at combinations of chlorine and exposure times commonly employed to reduce fouling. The problem appears to be particularly severe for the genus Acartia which constitute a major portion of the microfauna grazed upon by larval fish.

ICHTHIOPLANKTON

Information on the tolerance of larval and juvenile fish indicate that this group is also very sensitive to chlorine. Two species were investigated at our laboratory, the winter flounder (Pseudopleuronectes americanus) and the yellowtail flounder (Limanda ferruginea). The results indicate that the winter flounder larvae were in the same sensitivity range as the copepods. Twenty-four hour exposures of the yellowtail flounder produced an LC-50 of 0.2 ppm and 0.10 ppm on two separate samples of larvae. Regretably, similar data was unavailable for the winter flounder. What is significant is that these values are comparable to the twenty-four hour exposure values derived from the microalgae. Alderson (19) studied the effects of low concentrations of free chlorine on the eggs and larvae of plaice (Pleuronectes platessa) (Table 5). Fairbanks et al. (20) in an investigation of chlorine

Table 5. THE CONCENTRATION OF CHLORINE (mgs/1) FATAL TO 50% OF PLEURONECTES PLATESSA LARVAE (19)

Chlorine (mgs/1)	Exposure time
0.028	96 hrs.
0.050	460 mins.
0.075	175 mins.
0.100	90 mins.
0.130	70 mins.

toxicity to young of the year menhaden (Brevoortia tyrannus) found

extensive mortalities at chlorine levels less than 1.0 ppm (Table 6). Comparing data from these studies reveals that the menhaden 60 minute TLm of 0.22 ppm is remarkably similar to the 70 minute TLm of 0.13 ppm for plaice larvae (19).

Table 6. THE TOXICITY OF CHLORINE TO YOUNG OF THE YEAR MENHADEN, BREVOORTIA TYRANNUS (20)

Exposure (min.)	LC_{50} (mgs/1)
10	0.70
15	0.64
30	0.27
60	0.22

These larval studies emphasize that short term exposure to chlorine levels of >0.1 ppm would produce a significant biological effect under routine intermittent dosage conditions. Of further concern is the potential damage to receiving water populations of these species inhabiting the environs of the discharge canal where discharge residuals often reach 0.1 ppm.

Holland et al. (10) investigated the toxicity of chlorine and chloramines to three species of salmon in seawater of 18 o/oo chlorinity between 6-9°C. Forty percent of Chinook salmon (365 day old) were killed after 24 hrs exposure to 0.05 ppm Cl_2. Pink salmon showed decreasing sensitivity to chlorine with age. Thirty-two day old pink salmon had a 72 hr LC-100 of 0.084 while 61 day old specimens had a 72 hr LC-50 of 0.91 with only 13% mortality noted after 72 hrs exposure at 0.13 ppm. Silver salmon showed complete mortality after 72 hrs exposure to 0.10 ppm. More recent studies on pink and Chinook salmon (21) demonstrate the interaction of temperature and chlorine. The most toxic effect for juveniles of these species occurred at a temperature of 10.0°C. The lethal time for 50% mortality ranged from 10 minutes at 0.5 mg/1 total residual for Chinook and 80 minutes at 0.5 mg/1 total residual for pink.

Though not originally related to a power plant problem, investigations on chlorine tolerance of juvenile spot (Leiostomus xanthurus) produced a 96 hr LC-50 of 0.09 mg/1 total residual chlorine. Estimated 24 and 6 hr LC-50's were 0.14 mg/1 and 0.28 mg/1 total

residual chlorine, respectively (22).

The data presented in this section provides an overview of chlorine toxicity to a wide variety of organisms that could be impacted by a power plant. The generalized summary presented in Fig. 5 shows the distinct response patterns of the major organism groups. By choosing any combination of chlorine concentration and exposure time one can determine what group or groups of organisms are to be affected. The data point represents fifty percent mortality at the respective concentration and exposure period. The response slope for the algae and copepods is quite similar though displaced while that of the ichthioplankton and juvenile fishes is much steeper and covers a wider range of concentrations. Obviously, intermittent chlorination which involves high residuals (<5.0 ppm) for exposure periods of up to 30 minutes will seriously affect phytoplankton, ichthioplankton, and zooplankton populations. Lower chlorine levels (i.e., 0.25) continuously applied would almost completely eliminate entrainment damage and produce discharge residuals <0.1 ppm thus protecting receiving waters. Still there is the potential hazard that continuous chlorination and its by-products (i.e., chlorinated organics) could impact larval and juvenile fish populations attracted by the plume.

FIELD VALIDATION--MORGANTOWN GENERATING STATION

The field and laboratory studies discussed above indicate that intermittent chlorination as generally practiced would result in serious damage to a variety of plankton organisms that would be entrained in cooling water. The laboratory studies, however, indicate that lower levels of chlorine (<0.25 ppm) may show only moderate damage to algae and be relatively safe for ichthioplankton and copepods if exposures did not exceed one hour. An opportunity to evaluate the effects of chlorine levels less than 1.0 ppm in the field arose and permitted us to verify laboratory studies. The primary objectives of this effort were to evaluate the biological impact of plant operational modes (particularly chlorination) as well as to complete the evaluation and verification of ATP as a field assessment of living biomass. This study was carried out with the cooperation of Martin-Marrietta and Potomac Electric Power Company.

Sampling stations were set up at the intake, discharge, and terminus of the canal. The passage of a unit water mass through the plant requires approximately 2 minutes with an additional 45 minutes to traverse the length of the canal. Studies were performed in late spring, May 29-June 6; summer, August 23-28; and September 19-20, 1973. A thermal load of 6°C was typical with chlorination levels of 0.55 and 0.32 ppm total residual. These residual values were determined at the point of dosing and were usually reduced to 0.2 and 0.1 within the canal. Laboratory studies would predict that the

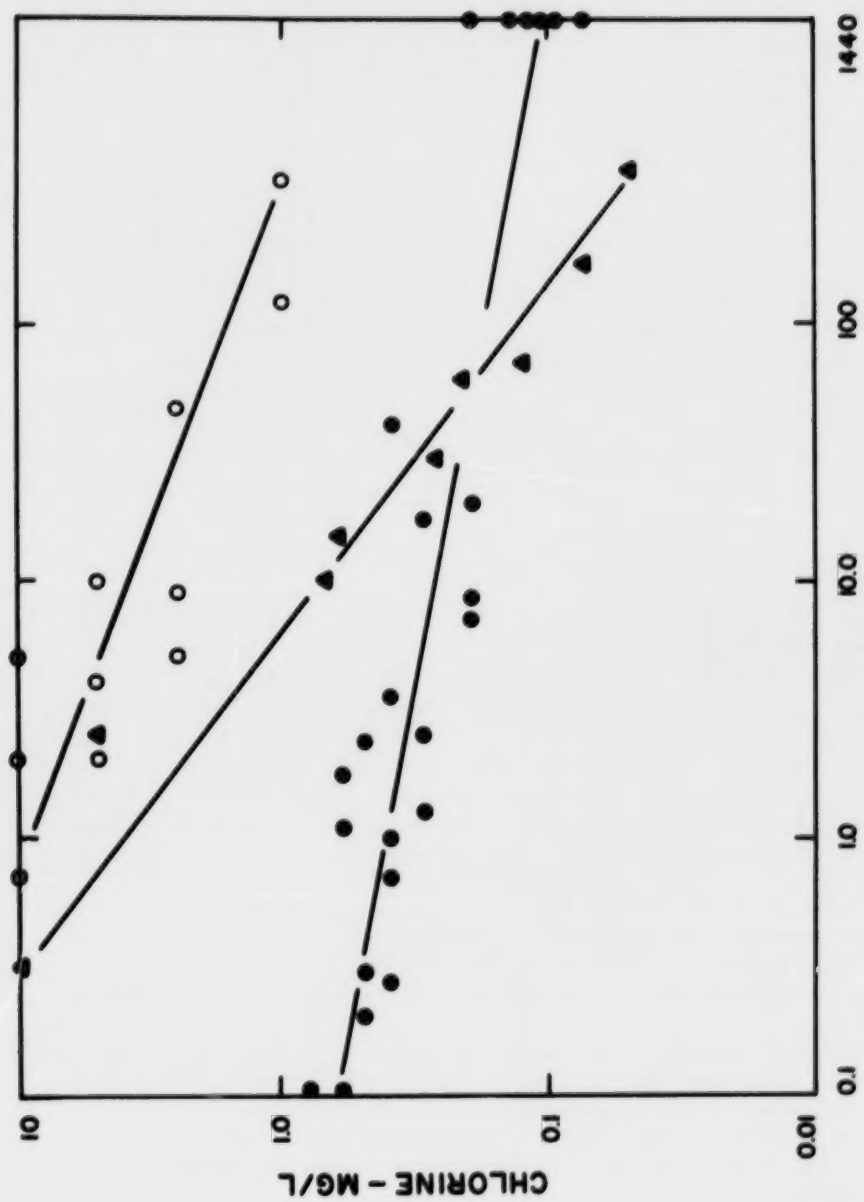

Figure 5. Distribution of 50% response isopleths for marine phytoplankton

higher dose level (0.55 ppm) would produce complete destruction of phytoplankton within the two minute plant transit time. The lower dose (0.32 ppm) should be effective within 10 minutes. The field exposures would also include an additional thermal stress component from plant passage. Temperatures ranged from 18-27°C which were somewhat higher than those of the laboratory studies.

The data indicate that both chlorination levels produced essentially complete destruction of phytoplankton ATP (Table 7). Since ATP is

Table 7. THE EFFECTS OF PLANT PASSAGE AND CHLORINATION TO ESTUARINE PHYTOPLANKTON[a]

Date	Intake	Discharge	Canal
5-29	0.43	0.38	0.38
5-30[b]	0.42	0.08	0.04
8-22	0.61	0.27	0.14
8-23[c]	0.43	0.03	0.03
8-28[c]	0.49	0.065	0.03
9-19	0.65	0.40	0.25
9-20[b]	0.30	0.14	0.10

[a]Tabular values are µgs ATP/liter of water.

[b]0.32 ppm Cl_2.

[c]0.55 ppm Cl_2.

rapidly lost upon cell death these results indicate irreversible loss of living biomass. Plant passage in the absence of chlorination resulted in no damage to pumped phytoplankton on 5-29, but show definite indications of damage on 8-22 and 9-19. The effects of chlorination are far more severe and appear to be independent of dosage. This corroborates the laboratory studies in that both levels produce severe damage. An effort was made to isolate the chlorination effects on 9-20 by removing a water sample immediately after chlorination and prior to thermal loading at the condensers. The samples were held for 2 and 45 minutes at ambient temperatures to

simulate exposure durations at the discharge and canal sampling points. The ATP value at the discharge was 0.16 and at the canal, 0.05 µgs ATP/liter. This indicated that the through plant damage was primarily attributable to chlorine.

Attempts to evaluate the effects of these two chlorine regimes on zooplankton were complicated by the unexpectedly high mortalities encountered from plant passage during non-chlorination periods. However, when rates of sinking were compared, the effects of chlorination were quite evident (Gentile, Lackie, and Cheer, 1974 [23]). The degree to which this response reflected irreversible damage due to chlorination alone could not be proven since mechanical damage was simultaneously occurring. When ATP/organism was evaluated there were no significant differences between chlorination and non-chlorination cycles. By the time the water mass had reached the end of the discharge canal, the damaged organisms had settled out of the water column and only healthy survivors were being sampled. Comparison of intake and canal stations by both ATP analysis and microscopic counts revealed a 50-15% loss of zooplankton biomass. This data supports recent findings by Carpenter et al., 1974 (24).

Investigating the effects of plant passage on productivity at a Long Island Power station, Carpenter et al. (25) observed a 70% decrease in productivity at 0.12 ppm continuous chlorination and 25% decrease at 0.2 ppm intermittent. From Table 7, a 30% decrease in carbon-14 assimilation was noted at 0.2 ppm after 5 minutes exposure. Post exposure growth rates, however, were normal leading to the conclusion that the noted effects on productivity might be transitory. The data from this study indicate that at 0.32 ppm damage was essentially complete and irreversible.

CONCLUSION

The results of field and laboratory investigations on chlorine toxicity to marine organisms reported here have been primarily developed from power plant related activities. These studies deal with chlorine primarily in the available form and are unlike municipal waste water systems in which a considerable percentage of the chlorine is in a combined state. Furthermore, the discharge of chlorinated water from power plants is of an intermittent nature rather than continuous as it is in municipal treatment plants. Although these differences are important in terms of application of this data for water quality standards, certain general conclusions emerge.

Chlorine is one of the few elements for which experimentation has demonstrated specific toxicity of a chemical to marine organisms. Phytoplankton, juvenile fish, fish larvae, all show demonstrable impairment of biological function at residual chlorine levels of

0.1 ppm. Oysters show reduced pumping rates at <0.05 ppm (26) and extensive mortalities to plaice larvae occur at 0.028 ppm after ninety-six hours exposure. Muchmore and Epel (27) demonstrated the spermicidal effect of 0.05 ppm available chlorine to three marine species of invertebrates. Holland et al. (10) has demonstrated that three species of young salmon are sensitive to chlorine at <0.1 ppm. Research on combined chlorine toxicity in freshwater systems indicate that these compounds are of comparable toxicity to available chlorine. Studies at this laboratory indicate that natural seawater chlorinated (10 ppm initially) for 100 hours and treated with sodium thiosulfate to eliminate any available chlorine was still inhibitory to phytoplankton growth.

In developing effluent guidelines and water quality criteria for chlorine discharges into the marine environment, a conservative approach is necessary. While considerable information is available for short term effects of available chlorine, little is known about the long term exposures that will result from treated wastes discharge through ocean outfalls. This is an area that deserves immediate research attention. Nevertheless, the data available does provide a sound scientific foundation for recommending limits on the concentration, form, and duration of chlorine discharges into marine waters.

In order to assure that indigenous marine populations are protected from irreversible damage from chlorine and its organic derivatives such as chlorinated amines, chlorinated phenols, and other potential unidentified chlorinated derivatives, levels of <u>total residual chlorine</u> should not exceed 0.01 mg/l for up to 2 hours in any 24 hour period. This value is in fact not a conservative number, since examination of Fig. 5 clearly demonstrates that 50% mortality and growth inhibition occur at levels of 0.075-0.10 ppm for this exposure time. These are not nebulous biological responses but mortality and impairment of reproductive function. Therefore, a margin of safety must be afforded the biota since continued loss of 50% of the entrained populations and jeopardizing receiving water populations is an unnecessary risk. Without adequate research into the chronic effects of continuous chlorination, water quality criteria for this type of discharge can only be inferred from studies performed in freshwater systems and from marine short term data.

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